CLAIMS

What is claimed is:

1. A method for monitoring the progress of fat loss in a patient during a weight loss program which comprises, contacting a body fluid sample from said patient with a solid test strip to provide a color indication of the presence in said body fluid of β-hydroxybutyrate, optionally together with acetoacetate and/or acetone.

2. The method according to claim 1, wherein said body fluid is a member selected from the group consisting of urine, blood, serum and saliva.

3. The method according to claim 1 or 2, wherein said solid test strip comprises:
   a) a support layer; and
   b) a reagent layer on said support layer, said reagent layer comprising:
      i) β-hydroxybutyrate dehydrogenase (β-HBD),
      ii) nicotinamide adenine dinucleotide (NAD),
      iii) a tetrazolium dye precursor, and
      iv) an electron mediator capable of transferring an electron to said dye precursor to effect a color change.

4. The method according to claim 3, wherein said β-HBD is an enzyme that is not inhibited by chloride ions.

5. The method according to claim 4, wherein said β-HBD is from *Pseudomonas* or *Alcaligenes*.

6. The method according to claim 3, wherein said electron mediator is a member selected from the group consisting of diaphorase, phenazinium methyl sulfate (PMS) and 1-methoxy-5-methylphenazinium methyl sulfate (1-methoxy PMS).

7. The method according to claim 1, wherein said tetrazolium dye precursor is a member selected from the group consisting of 2-(2'benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium (BSPT), 2-benzothiazolyl-(2)-3,5-diphenyl tetrazolium (BTDP), 2,3-di(4-nitrophenyl) tetrazolium (DNP), 2,5-diphenyl-3-(4-styrylphenyl)

tetrazolium (DPSP), distyryl nitroblue tetrazolium (DS-NBT), 3,3'-[3,3'-dimethoxy-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(4-nitrophenyl)-5-ph enyl(-2H tetrazolium (NBT), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium (MTT), 2-phenyl-3-(4-carboxyphenyl)-5-methyl tetrazolium (PCPM), tetrazolium blue (TB), thiocarbamyl nitroblue tetrazolium (TCNBT), tetranitroblue tetrazolium (TNBT), tetrazolium violet, (TV), 2-benzothiazothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcar bamoyl)phenyl]-2H-tetrazolium (WST-4), and 2,2'-dibenzothiazolyl-5,5'-bis[4-di(2-sulfoethyl)carbamoylphenyl]-3,3'-(3, 3'-dimethoxy- 4,4'-biphenylene)ditetrazolium, and disodium salt (WST-5).

8. The method according to claim 6, wherein said diaphorase is a lipoic dehydrogenase, a ferredoxin-NADP reductase or a lipoamide dehydrogenase.

9. A method of assaying for β-hydroxybutyrate and acetoacetate in a sample which comprises:

b) contacting a sample with a composition comprised of β-hydroxybutyrate dehydrogenase (β-HBD) and nicotinamide adenine dinucleotide (NAD) at a pH of less than 8.5, whereby

(iv) β-hydroxybutyrate (β-HB) reacts with NAD to produce acetoacetate and reduced-type nicotinamide adenine dinucleotide (NADH),

(v) a portion of the NADH produced in (i) reacts with acetoacetate in the presence of β-HBD to produce β-HB, and

(vi) a portion of the NADH produced in (i) is converted into a colored product,; and

c) detecting the presence of said colored product.

10. The method according to claim 9, wherein said sample is a body fluid from a patient.

11. The method according to claim 10, wherein said body fluid is a member selected from the group consisting of urine, blood, serum and saliva.

12. The method according to claim 9, wherein said β-HBD is an enzyme that is not inhibited by chloride ions.

13. The method according to claim 9, wherein said β-HBD is from *Pseudomonas* or *Alcaligenes*.

14. The method according to claim 10, wherein said composition further comprises a tetrazolium dye precursor and an electron mediator selected from the group consisting of diaphorase, phenazinium methyl sulfate (PMS) and 1-methoxy-5-methylphenazinium methyl sulfate (1-methoxy PMS), whereby said NADH is converted into a colored product by reacting with a tetrazolium dye precursor in the presence of said electron mediator to produce reduced tetrazolium dye, and the detected color product is said reduced tetrazolium dye.

15. The method according to claim 14, wherein said tetrazolium dye precursor is a member selected from the group consisting of 2-(2'benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium (BSPT), 2-benzothiazolyl-(2)-3,5-diphenyl tetrazolium (BTDP), 2,3-di(4-nitrophenyl) tetrazolium (DNP), 2,5-diphenyl-3-(4-styrylphenyl) tetrazolium (DPSP), distyryl nitroblue tetrazolium (DS-NBT), 3,3'-[3,3'-dimethoxy-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(4-nitrophenyl)-5-ph enyl(-2H tetrazolium (NBT), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium (MTT), 2-phenyl-3-(4-carboxyphenyl)-5-methyl tetrazolium (PCPM), tetrazolium blue (TB), thiocarbamyl nitroblue tetrazolium (TCNBT), tetranitroblue tetrazolium (TNBT), tetrazolium violet, (TV), 2-benzothiazothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcar bamoyl)phenyl]-2H-tetrazolium (WST-4), and 2,2'-dibenzothiazolyl-5,5'-bis[4-di(2-sulfoethyl)carbamoylphenyl]-3,3'-(3, 3'-dimethoxy- 4,4'-biphenylene)ditetrazolium, and disodium salt (WST-5).

16. The method according to claim 14, wherein said diaphorase is a lipoic dehydrogenase, a ferredoxin-NADP reductase or a lipoamide dehydrogenase.

17. The method according to claim 9, wherein said assaying is conducted to monitor fat loss in a patient during a weight loss program.

18. The method according to claim 9, wherein said assaying is conducted in the treatment of a disease selected from the group consisting of diabetes, cardiovascular disorder and epilepsy.

19. A test strip for assaying for β-hydroxybutyrate, and optionally acetoacetate in a sample comprising:

c) a support layer; and

d) a reagent layer on said support layer, said reagent layer comprising:

iv) β-hydroxybutyrate dehydrogenase (β-HBD),

v) nicotinamide adenine dinucleotide (NAD),

vi) a tetrazolium dye precursor, and

vii) an electron mediator.

20. The test strip according to claim 19, wherein said β-HBD is an enzyme that is not inhibited by chloride ions.

21. The test strip according to claim 19, wherein said β-HBD is from *Pseudomonas* or *Alcaligenes*.

22. The method according to claim 19, wherein said electron mediator is a member selected from the group consisting of diaphorase, phenazinium methyl sulfate (PMS) and 1-methoxy-5-methylphenazinium methyl sulfate (1-methoxy PMS).

23. The test strip according to claim 19, wherein said tetrazolium dye precursor is a member selected from the group consisting of 2-(2'benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium (BSPT), 2-benzothiazolyl-(2)-3,5-diphenyl tetrazolium (BTDP), 2,3-di(4-nitrophenyl) tetrazolium (DNP), 2,5-diphenyl-3-(4-styrylphenyl) tetrazolium (DPSP), distyryl nitroblue tetrazolium (DS-NBT), 3,3'-[3,3'-dimethoxy-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(4-nitrophenyl)-5-ph enyl(-2H tetrazolium (NBT), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium (MTT), 2-phenyl-3-(4-carboxyphenyl)-5-methyl tetrazolium (PCPM), tetrazolium blue (TB), thiocarbamyl nitroblue tetrazolium (TCNBT), tetranitroblue tetrazolium (TNBT), tetrazolium

violet, (TV), 2-benzothiazothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcar bamoyl)phenyl]-2H-tetrazolium (WST-4), and 2,2'-dibenzothiazolyl-5,5'-bis[4-di(2-sulfoethyl)carbamoylphenyl]-3,3'-(3, 3'-dimethoxy- 4,4'-biphenylene)ditetrazolium, and disodium salt (WST-5).

24. The test strip according to claim 22, wherein said diaphorase is a lipoic dehydrogenase, a ferredoxin-NADP reductase or a lipoamide dehydrogenase.

25. The test strip according to claim 19, wherein said β-HBD is contained in said reagent layer in an amount of 1 or more Units per test strip, wherein 1 Unit of said enzyme is equal to an amount which will oxidize 1 μ mole of substrate at a pH 8.5 of 30° C.

26. The test strip according to claim 19, wherein said reagent layer further comprises a buffer in an amount such that the pH of said reagent layer is 8.5 or less.

27. A method for assaying total ketone bodies including β-hydroxybutyrate, acetoacetate and acetone in a sample which comprises,

(a) contacting a sample with a solid test strip which comprises

i) a support layer; and

ii) a reagent layer on said support layer, said reagent layer comprising β-hydroxybutyrate dehydrogenase (β-HBD), nicotinamide adenine dinucleotide (NAD), and a detector selected from nitropruside and a diazonium salt, whereby β-HB reacts with NAD to produce acetoacetate and said acetoacetate reacts with said detector to produce a color change; and

(b) detecting the presence of said color change.

28. The method according to claim 27, wherein said diazonium salt is 4-nitrobenzene diazonium tetrafluoroborate.

29. The method according to claim 27, wherein said sample is a body fluid from a patient.

30. The method according to claim 29, wherein said body fluid is a member selected from the group consisting of urine, blood, serum and saliva.

31. The method according to claim 27, wherein said assaying is conducted to monitor fat loss in a patient during a weight loss program.

32. The method according to claim 27, wherein said assaying is conducted in the treatment of a disease selected from the group consisting of diabetes, cardiovascular disorder and epilepsy.

33. A solid test strip for assaying for total ketone bodies including β-hydroxybutyrate, acetoacetate, and acetone in a sample which comprises:

(a) a support layer; and

(b) a reagent layer on said support layer, said reagent layer comprising:

(i) β-hydroxybutyrate dehydrogenase (β-HBD),

(ii) nicotinamide adenine dinucleotide (NAD),

(iii) nitroprusside or a diazonium salt.

34. The test strip according to claim 33, wherein said β-HBD is an enzyme that is not inhibited by chloride ions.

35. The test strip according to claim 33, wherein said β-HBD is from *Pseudomonas* or *Alcaligenes*